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No Genetic Differentiation between *Asarum maculatum* and *A. sieboldii* var. *sieboldii* (Aristolochiaceae) in Korea

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Asarum maculatum Nakai, a perennial herb endemic to southern Korea, is unique in its variegated leaves. However, the independence of this species is doubtful because the species co-occurs with *A. sieboldii* Miq. var. *sieboldii* in the same geographical area in Korea, and no significant difference is found between the two taxa in external morphology, except for leaf variegation. We examined the genetic differentiation between *A. maculatum* and *A. sieboldii* var. *sieboldii* using enzyme electrophoresis. The genetic differentiation between the two taxa in the same locality was not greater than that among the intraspecific populations. Moreover, there is little genetic differentiation between the mixed populations of the two taxa and the populations composed only of *A. sieboldii* var. *sieboldii*. Consequently, *A. maculatum* is not differentiated genetically from *A. sieboldii* var. *sieboldii*, and the two taxa should be regarded as the same species. Hence, a new combination, *A. sieboldii* f. *maculatum* (Nakai) Yamaji (comb. et stat. nov.), is proposed.

Key words: Aristolochiaceae, *Asarum maculatum*, *A. sieboldii* var. *sieboldii*, enzyme electrophoresis, genetic distance, Korea, taxonomic status, variegated leaf

Asarum maculatum Nakai is a perennial herb endemic to southern Korea (Nakai 1914, Maekawa 1936, 1956, Lee 1979, Lee & Lee 200) and has been characterized by having comparatively thick and variegated leaves (Nakai 1914, Maekawa 1936). In gross morphology, the species is supposed to be closely related to *A. sieboldii* Miq., which is widely distributed from Korea to Japan (Honshu and Kyushu) and in southern China (Maekawa 1936, Satake & Momiyama 1982, Cheng & Yang 1983, Yamaki *et al.* 1996). In a recent taxonomic paper on Korean *Asarum*, Yamaki *et al.* (1996) proposed two varieties for *A. siebodii*, namely var. *sieboldii* and var. *versicolor* Yamaki. More recently, as noted by Lee & Lee (2000), *A. sieboldii* var. *versicolor* is regarded as a distinct species separated from *A. sieboldii* var. *sieboldii* as well as *A. maculatum* based on its comparatively slender calyx tube and smaller calyx lobe. In this study therefore we are concerned with *A. sieboldii* var. *sieboldii* for comparison with *A. maculatum*.

Although *Asarum maculatum* often co-occurs with *A. sieboldii* var. *sieboldii* in some localities, especially in southern Korea, the two taxa have generally been separated from each other without difficulty by leaf thickness and leaf variegation.

Nevertheless, as noted by Yamaki *et al.* (1996), the above two taxa were very similar, and their quantitative floral traits highly overlapped each other. Furthermore, throughout our preliminary observation we could not find any differences between them in leaf thickness and in some floral characters (Yamaji unpublished). Accordingly, it is doubtful whether *A. maculatum* is genetically independent from *A. sieboldii* var. *sieboldii*. In this study we employed enzyme electrophoresis to examine genetic differentiation between *A. maculatum* and *A. sieboldii* var. *sieboldii*, and incorporated the results into a taxonomic consideration of them.

Materials and Methods

Sample collections

A total of four populations of *Asarum maculatum* were examined covering almost whole area in which this species is distributed. To compare with "pure" *A. sieboldii* var. *sieboldii*, four populations composed only of *A. sieboldii* var. *sieboldii* were examined.

For outgroups, two populations of *Asarum patens* (Yamaki) Y. N. Lee were also employed. The localities of populations examined are shown in Fig. 1 and Table 1. A few individuals from each population were deposited in the Herbarium of Tohoku University (TUS) as dried voucher specimens. For enzyme electrophoresis, we collected one leaf from each individual and transported it in an icebox. The samples were kept in a refrigerator for up to three weeks until electrophoresis was carried out. At three weeks after sampling, enzyme activity did not decrease.

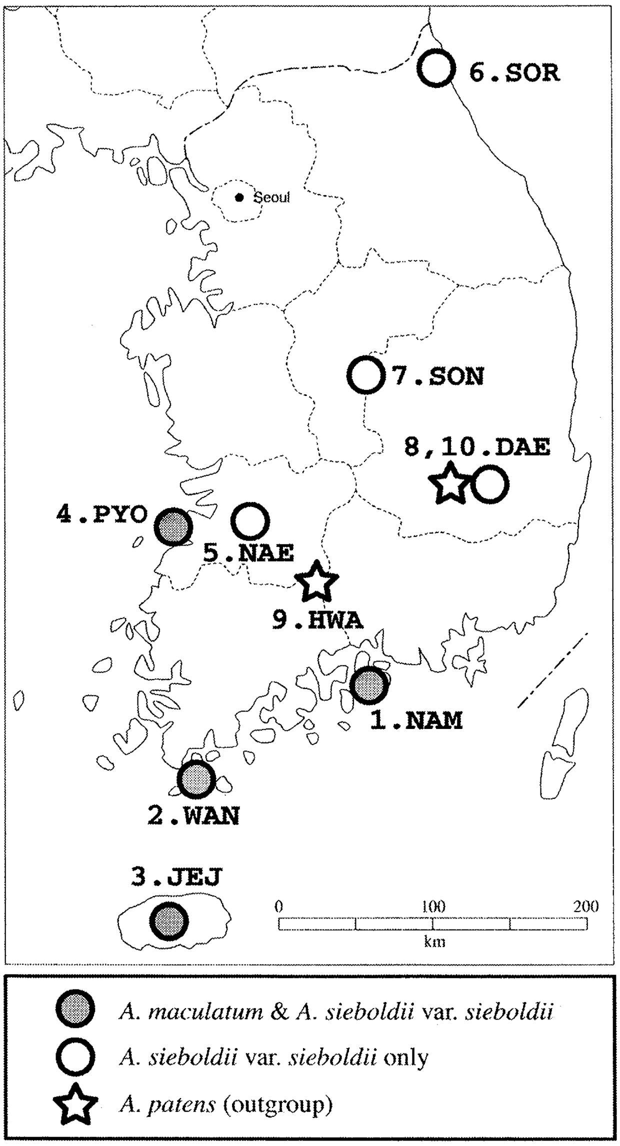

FIG. 1. Populations examined in this study. Mixed populations of *Asarum maculatum* and *A. sieboldii* var. *sieboldii*: gray circle, populations composed only of *A. sieboldii* var. *sieboldii* : open circle, *A. patens* (outgroup) populations: star.

Gel electrophoresis

Two hundred milligrams of a leaf sample were homogenized in 2 ml of an extract buffer made up of 93 mM Tris-HCI (pH 8.0), 23.4% glycerol, 0.6% (v/v) Tween 80, 2.8 mM EDTA, 2.3 mM NAD, 1.6 mM NADP, 11 mM dithiothreitol, 0.5% 2-mercaptoethanol, 0.08% bovine serum albumin, and 7% poly-vinyl-polypyrolidone (Uchida *et al.* 1991). The homogenates were centrifuged at 15000 rpm at 4°C for 30 min. The resultant supernatant was used for crude extract of the enzymes.

Polyacrylamide vertical slab gel electrophoresis was carried out according to the procedures described by Davis (1964) and Ornstein (1964). Ten microlitres of the crude extract of enzyme were used for electrophoresis for each enzyme. The fol-

TABLE 1. The locality, number of individuals of each taxon in each population. Abbreviation name of each population is in parentheses. "HY" in voucher is abbreviation of "H. Yamaji".

No.	Locality	Ind. no (code of pop.)	voucher	total
Mixed populations of *Asarum maculatum* and *A. sieboldii* var. *sieboldii*				
1	Gyeongsangnam-do, Namhaedo			32
	variegated (*A. maculatum*)	15 (M-NAM)	*T. Shinkawa in 2000*	
	non-variagated (*A. sieboldii* var. *sieboldii*)	17 (S-NAM)	*T. Shinkawa in 2000*	
2	Jeollanam-do, Wando Is.			32
	variegated (*A. maculatum*)	12 (M-WAN)	*HY et al. 8065*	
	non-variagated (*A. sieboldii* var.*sieboldii*)	20 (S-WAN)	*HY et al. 8067*	
3	Jeju-do, Mt. Hallasan			24
	variegated (*A. maculatum*)	12 (M-JEJ)	*HY et al. 8140*	
	non-variagated (*A. sieboldii* var.*sieboldii*)	12 (S-JEJ)	*HY et al. 8141*	
4	Jeollabuk-do, Pyonsanbando			12
	variegated (*A. maculatum*)	10 (M-PYO)	*HY et al. 8070*	
	non-variagated (*A. sieboldii* var.*sieboldii*)	2 (S-PYO)	*HY et al. 8071*	
Populations composed only of *A. sieboldii* var. *sieboldii*				
5	Jeollanam-do, Naejangsan National Park	10 (S-NAE)	*HY et al. 8074*	10
6	Gangwon-do, Soraksan Nationakl Park	6 (S-SOR)	*HY et al. 8170*	6
7	Chungcheongbuk-do, Songnisan National Park	12 (S-SON)	*HY et al. 8095*	12
8	Daegu City, Mt. Apsan	7 (S-DAE)	*HY et al. 8090*	7
Outgroup (*A. patens*)				
9	Jeollanam-do, ,Hwaomsa	4 (P-HWA)	*HY et al. 8078*	4
10	Daegu City, Mt. Apsan	3 (P-DAE)	*HY et al. 8085*	3

lowing 12 enzyme systems were examined: alcohol dehydrogenase (ADH), diaphorase (DIA), fructose-1,6-diphosphatase (F1,6DP), glucose-6-phosphate 1-dehydrogenase (G6PD), glutamate dehydrogenase (GDH), leucyl aminopeptidase (LAP), malate dehydrogenase (MDH), menadione reductase (MNR), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6PGD), shikimate dehydrogenase (SKDH).

Statistical analysis

Allele frequencies in each population of the taxa were calculated for the loci encoding the 12 enzyme systems. The following indices were calculated manually and were used to quantify the amount of genetic diversity within each population or each taxon: proportion of polymorphic loci (P), mean number of alleles per locus (A), the number of alleles per polymorphic loci (AP), and expected heterozygosity (h).

Genetic identity values (I) and genetic distance values (D) for each pairwise comparison of all populations examined were calculated manually (Nei 1972). We obtained a phenogram based on the standard genetic distance (D) using the UPGMA method (Sneath & Sokal 1973) on MEGA version 2.1 (Kumar *et al.* 2001). We also calculated pairwise *Fst* for the populations of *Asarum maculatum* and *A. sieboldii* var. *sieboldii*, and tested the significance of the differentiation between each pair of populations using FSTAT version 2.9.3 (Goudet 2001).

Results and Discussion

Genetic diversity within populations of Asarum maculatum *and* A. sieboldii *var.* sieboldii

Thirteen loci were resolved for all of three taxa. At the population level, nine (69%), eight (62%), and six (46%) loci were polymorphic for the populations of *Asarum maculatum* and *A. sieboldii* var. *sieboldii*, the populations of *A. maculatum*, and the populations of *A. sieboldii* var. *sieboldii*, respectively. Between *A. maculatum* + *A. sieboldii* var. *sieboldii* and the outgroup, *A. patens*, the same alleles were shared in 10 loci. Only one locus was polymorphic in *A. patens*. Genetic diversity within a population indicated in resultant values of *P*, *A*, *AP*, and *h* (Table 2). These values were in similar level in *A. maculatum* and *A. sieboldii* var. *sieboldii.*

Genetic differentiation between Asarum maculatum *and* A. sieboldii *var.* sieboldii

Table 3 summarizes the genetic identity (*I*) and genetic distance (*D*) between the populations. Mean

TABLE 2. Genetic diversity at the population and species level of three species of *Asarum* in Korea. P = proportion of polymorphic loci (%), A = mean number of alleles per locus, AP = the number of alleles per polymorphic loci, and h = expected heterozygosity.

	P	*A*	*AP*	*h*
Mixed populations of *A. maculatum* and *A. sieboldii* var. *sieboldii*				
M-NAM	23.1	1.23	2.00	0.046
S-NAM	15.4	1.15	2.00	0.066
M&S-NAM	23.1	1.23	2.00	0.060
M-WAN	30.8	1.31	2.00	0.078
S-WAN	30.8	1.38	2.25	0.086
M&S-WAN	46.2	1.54	2.17	0.085
M-JEJ	00.0	1.00	-	0.000
S-JEJ	7.7	1.08	2.00	0.021
M&S-JEJ	7.7	1.08	2.00	0.012
M-PYO	23.1	1.31	2.33	0.087
S-PYO	7.7	1.08	2.00	0.038
M&S-PYO	23.1	1.31	2.33	0.084
Mean of mixed populations	25.0	1.29	2.13	0.060
Populations composed only of *A. sieboldii* var. *sieboldii*				
S-NAE	30.8	1.38	2.25	0.083
S-SON	15.4	1.23	2.50	0.048
S-SOR	7.7	1.15	3.00	0.038
S-DAE	15.4	1.15	2.00	0.050
Mean of uniform populations	17.3	1.23	2.44	0.055
Mean of *A. maculatum*	19.2	1.21	2.11	0.053
Mean of *A. sieboldii* var. *sieboldii*	16.3	1.20	2.25	0.054
Outgroups				
P-HWA	7.70	1.08	2.00	0.029
P-DAE	7.70	1.08	2.00	0.034

TABLE 3. Genetic identity values (*I*: upper right) and genetic distance values (*D*: lower left) between populations.

	M-NAM	S-NAM	M-WAN	S-WAN	M-JEJ	S-JEJ	M-PYO	S-PYO	S-NAE	S-SON	S-SOR	S-DAE	P-HWA	P-DAE
M-NAM	-	0.993	0.980	0.976	0.995	0.994	0.903	0.896	0.996	0.990	0.988	0.991	0.447	0.673
S-NAM	0.007	-	0.958	0.961	0.976	0.977	0.880	0.874	0.988	0.970	0.969	0.978	0.626	0.661
M-WAN	0.021	0.043	-	0.995	0.985	0.986	0.934	0.916	0.983	0.978	0.977	0.985	0.656	0.666
S-WAN	0.024	0.039	0.005	-	0.975	0.972	0.939	0.910	0.980	0.969	0.967	0.977	0.653	0.643
M-JEJ	0.005	0.024	0.015	0.025	-	0.998	0.910	0.902	0.992	0.995	0.994	0.992	0.644	0.678
S-JEJ	0.006	0.024	0.014	0.028	0.002	-	0.906	0.899	0.993	0.993	0.991	0.994	0.651	0.686
M-PYO	0.102	0.128	0.069	0.063	0.095	0.099	-	0.985	0.918	0.921	0.902	0.913	0.583	0.579
S-PYO	0.110	0.135	0.088	0.094	0.103	0.107	0.015	-	0.907	0.914	0.893	0.895	0.567	0.572
S-NAE	0.004	0.013	0.018	0.021	0.008	0.008	0.086	0.097	-	0.988	0.984	0.997	0.649	0.676
S-SON	0.011	0.031	0.022	0.031	0.005	0.007	0.082	0.089	0.012	-	0.997	0.987	0.663	0.699
S-SOR	0.012	0.032	0.023	0.034	0.006	0.009	0.103	0.113	0.016	0.003	-	0.985	0.670	0.705
S-DAE	0.009	0.023	0.015	0.023	0.008	0.006	0.091	0.111	0.003	0.014	0.015	-	0.419	0.685
P-HWA	0.805	0.468	0.422	0.427	0.440	0.429	0.539	0.567	0.432	0.411	0.400	0.658	-	0.947
P-DAE	0.396	0.413	0.407	0.441	0.388	0.377	0.547	0.559	0.392	0.359	0.349	0.379	0.055	-

pairwise *I* between populations of *Asarum maculatum* and *A. sieboldii* var. *sieboldii* in the same locality was obviously larger than that between populations of *A. maculatum* and that between populations of *A. sieboldii* var. *sieboldii* (Table 4). Fig. 2 is a phenogram constructed using the UPGMA method based on the genetic distance (*D*). Populations of each taxon were not placed in the same cluster. On the other hand, three of four pairs of populations of *A. maculatum* and *A. sieboldii* var. *sieboldii* in the same locality were in the same clusters. In addition, four populations of *A. sieboldii* var. *sieboldii* which do not co-occur with *A. maculatum* were placed in clusters of the mixed populations.

The *Fst* values for all four pairs of populations of the two taxa from the same locality were comparatively lower than those of the other pairs (Table 5). Moreover, they are not significantly different in standard Bonferroni corrections at the 5% nominal level (Goudet 2001).

Those results indicate that no genetic gap is found between *Asarum maculatum* and *A. sieboldii* var. *sieboldii* examined in this study.

Taxonomic status of Asarum maculatum

As mentioned above, there is no genetic differentiation between *Asarum maculatum* and *A. sieboldii* var. *sieboldii* occurring in Korea. The conspicuous leaf variegation, which is probably the only diagnostic character in these taxa, is merely intraspecific polymorphism, as in some other species of *Asarum* sect. *Heterotropa*: leaf variegation varies considerably in a single species or even within a population (Satake & Momiyama 1982). Moreover, in the course of our sample collections, we recognized little or no discrete difference between *A. maculatum* and *A. sieboldii* var. *sieboldii*, and between the mixed populations of *A. maculatum* and *A. sieboldii* var. *sieboldii* in other floral and leaf morphology. Yamaki *et al.* (1996) also recognized that floral quantitative characters are highly overlapped

TABLE 4. Mean values and the range for Nei's (1972) genetic identity (*I*) and standard genetic distance (*D*) for pairwise combination in *Asarum maculatum* and *A. sieboldii* var. *sieboldii* in Korea.

	I		*D*	
	Mean	Range	Mean	Range
A. maculatum* x *A. sieboldii* var. *sieboldii	0.993	0.985 - 0.998	0.007	0.002 - 0.015
within single population				
within taxa				
A. maculatum	0.951	0.903 - 0.995	0.050	0.005 - 0.102
A. sieboldii var. *sieboldii* in mixed populations	0.932	0.874 - 0.977	0.070	0.024 - 0.135

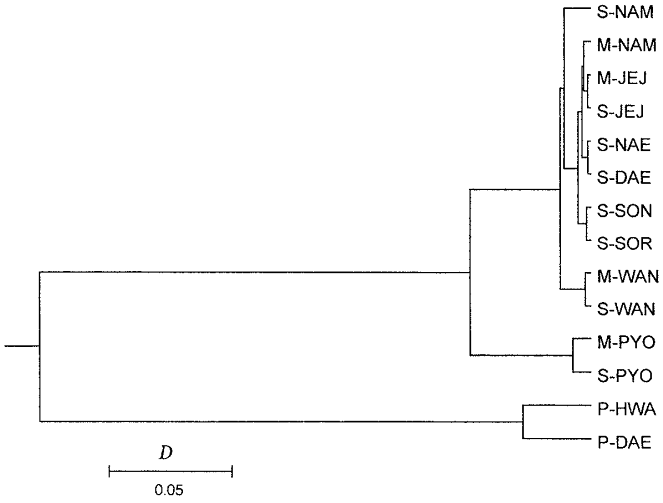

FIG. 2. Phylogram for three taxa of *Asarum* in Korea using the UPGMA method on Nei's (1972) standard genetic distance (*D*).

TABLE 5. *Fst* values between populations. Values with underbar indicates *Fst* values between *Asarum maculatum* and *A. sieboldii* var. *sieboldii* in the same locality. Asterisk indicates that they are significantly different in standard Bonferroni corrections at the 5% nominal level (Goudet 2001).

	S-NAM	M-WAN	S-WAN	M-JEJ	S-JEJ	M-PYO	S-PYO	S-NAE	S-SON	S-SOR	S-DAE
M-NAM	0.091	0.344	0.545*	0.287	0.261*	0.660*	0.946	0.132	0.052	0.242	0.210*
S-NAM	-	0.288	0.478*	0.241	0.251*	0.602*	0.774	0.068	0.066	0.135	0.060*
M-WAN		-	0.035	0.326*	0.249*	0.574*	0.624	0.102	0.269	0.265	0.225*
S-WAN			-	0.480*	0.376*	0.634*	0.673	0.250	0.457*	0.444	0.383
M-JEJ				-	0.007	0.414*	0.529	0.117	0.205	0.210	0.105*
S-JEJ					-	0.324*	0.353	0.119	0.222*	0.215	0.140*
M-PYO						-	-0.066	0.422*	0.501*	0.513*	0.470
S-PYO							-	0.455	0.610	0.651	0.567
S-NAE								-	0.073	0.094	-0.064
S-SON									-	-0.018	0.101*
S-SOR										-	0.103

between the two taxa. Consequently, the two taxa should be regarded as the same species. We therefore classified the individuals of *A. maculatum* into a forma of *A. sieboldii* var. *sieboldii*, because the two taxa can not be differentiated genetically, and the individuals with variegated leaves occur sporadically within a population composed of individuals with non-variegated leaves in the different habitats studied.

Taxonomic Treatment

Asarum sieboldii Miq. f. **maculatum** (Nakai) Yamaji **comb. et stat. nov.**

Asarum maculatum Nakai, in Fedde, Repert. Sp. Nov. Regn. Veget. 13: 267. 1914; Nakai, Bot. Mag. Tokyo 28: 519. 1914; Matsumura, Shokubutsu-Mei - I, rev. et enlarg. ed., II: 48. 1916; Mori, Enum. Corean Pl. p. 128. 1922; T. Lee, Illustrated flora of Korea. p. 296. 1979; Y. Lee, Bulletin of Korea Plant Research 1: 20. 2000. TYPE: Korea, Jeju-do, Mt. Hallasan, 10 May 1913, *Nakai s. n.* (lectotype designated here: TI!, with a description “sp. nov.”; isolectotype: TI!, without a description “sp. nov.”).

Asiasarum maculatum (Nakai) F. Maek., in T. Nakai (ed.), Flora Sylvatica Koreana XXI: 18. 1936.

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